Published in final edited form as:
Cancer Epidemiol Biomarkers Prev. 2010 August ; 19(8): 2043–2054. doi: 10.1158/1055-9965.EPI-10-0233.

Melanocytic nevi, nevus genes and melanoma risk in a large case-control study in the United Kingdom

Julia A Newton-Bishop,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Yu-Mei Chang,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Mark M Iles,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

John C Taylor,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Bert Bakker,
Laboratory for Diagnostic Genome Analyses (LDGA), Department of Human and Clinical Genetics, Leiden University Medical Center, Netherlands, PO Box 9600, 2300RC Leiden

May Chan,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Susan Leake,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Birute Karpavicius,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Sue Haynes,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Elaine Fitzgibbon,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Faye Elliott,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Peter A. Kanetsky,

Corresponding author Professor Julia Newton Bishop, Professor of Dermatology, Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, St James's Hospital, Beckett Street, Leeds LS9 7TF, UK, Tel +44 113 2064573, Fax +44 113 2340183, j.a.newton-bishop@leeds.ac.uk.

Center for Clinical Epidemiology and Biostatistics, University of Pennsylvania, Philadelphia, USA 19104

Mark Harland,
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Jennifer H Barrett, and
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

D Timothy Bishop
Section of Epidemiology and Biostatistics, Leeds Institute of Molecular Medicine, University of Leeds, Leeds, UK LS97TF

Abstract

Background—Increased number of melanocytic nevi is a potent melanoma risk factor. We have carried out a large population-based case-control study to explore the environmental and genetic determinants of nevi and the relationship with melanoma risk.

Methods—We report nevus phenotype in relation to differing patterns of sun exposure, inherited variation at loci shown in recent genome-wide association studies to be nevus genes, and risk.

Results—Increased numbers of nevi were associated with holiday sun exposure, particularly on intermittently sun-exposed body sites (test for trend $p<0.0001$). Large nevi were also associated with holiday sun exposure ($p=0.002$). Single nucleotide polymorphisms (SNPs) on chromosomes 9 and 22 were associated with increased numbers of nevi ($p=0.04$ and $p=0.002$ respectively) and larger nevi ($p=0.03$ and $p=0.002$), whereas that on chromosome 6 was associated only with large nevi ($p=0.01$). Melanoma risk was associated with increased nevus count, large nevi and atypical nevi for tumors in all body sites (including rare sites) irrespective of age. The risk persisted when adjusted for inheritance of nevus SNPs.

Conclusions—The at-risk nevus phenotype is associated with behaviors known to increase melanoma risk (holiday sun exposure). Although SNPs on chromosomes 6, 9 and 22 were shown to be nevus genes they explained only a small proportion of melanoma risk and nevus phenotype; therefore a number of nevus genes likely remain to be identified.

Impact—This paper confirms the importance of nevi in melanoma pathogenesis and increases understanding of their genetic determinants.

Introduction

Cutaneous melanoma is predominantly a cancer of pale-skinned peoples. Within those pale-skinned populations, individuals with skin which tends to burn in the sun are at increased risk (1). The genetic basis of this increased susceptibility is becoming increasingly understood as a result both of candidate gene approaches, which resulted in the identification of variation in the melanocortin 1 receptor (*MC1R*) as a susceptibility gene (2, 3), and of genome-wide association studies, which identified the tyrosinase gene and the *ASIP* locus as common susceptibility genes (4, 5) (6). Sunburn and intermittent (holiday) sun exposure are the dominant environmental determinants of melanoma risk (rather than large cumulative exposures) in temperate climes, with stronger evidence for both intermittent sun exposure and cumulative exposures in hotter countries (7).

Although sun-sensitive characteristics are common at-risk phenotypes, the most potent phenotypic risk factor for melanoma is the presence of increased numbers of melanocytic nevi (8, 9). Melanocytic nevi are benign proliferations of melanocytes which are postulated

to result from sun-induced mutations in oncogenes, typically *BRAF* (10) and less frequently *NRAS* (11). In the majority of such neoplasms, subsequent melanocyte senescence is induced by tumor suppressor proteins such as p16, and the nevus therefore ceases to grow and becomes stable or even involutes (12). In a proportion of individuals, however, a greater number of nevi develop, and commonly the melanocytes continue to proliferate for longer before senescence is induced. Therefore these individuals often have bigger nevi (5mm or more in diameter), and those individuals with a particularly large number of nevi are said to have the dysplastic nevus or atypical mole syndrome. Twin studies have provided strong evidence that the number of nevi is predominantly genetically determined (13-16), with a smaller effect of sun exposure (17). Genome-wide association studies have recently identified a number of loci which determine nevus number (Duffy et al in press) (6, 18).

We report here a large case-control study of melanoma performed in the north of England, in which the risk associated with nevus phenotype was investigated in relation to patterns of sun exposure and the inheritance of 3 single nucleotide polymorphisms (SNPs) on chromosomes 6, 9 and 22, in previously identified nevus genes (18) (Duffy et al paper in press).

Materials and methods

Ascertainment of cases and controls

Studies were approved by the UK Multi-Centre Research Ethics Committee (MREC), and the Patient Information Advisory Group (PIAG). Population-ascertained incident melanoma cases were recruited to a case-control study in a geographically defined area of Yorkshire and the Northern region of England (67% participation rate) as has been described elsewhere (19). All patients gave written informed consent to participation. A total of 960 patients (aged between 18 and 76 years) were diagnosed in the period from September 2000 to December 2005 (19). The cases were identified via clinicians, pathology registers and the Northern and Yorkshire Cancer Registry to ensure maximal ascertainment. During the study, a pragmatic approach was adopted to recruitment: during two time periods patients with very thin tumors who were unlikely to contribute to information on the determinants of outcome were excluded given limited resources. During other time periods, all patients with invasive melanoma were eligible in order to sample the whole melanoma population for studies on aetiology. Thus between September 2000 and December 2001, and from July 2003 to December 2005, patients with Breslow thickness less than 0.75mm were not invited to participate. Between January 2002 and June 2003 all patients with invasive melanoma were invited to participate.

The 513 population-ascertained controls were identified by the cases' family doctors as not having cancer, and were randomly invited from individuals with the same sex and within the same 5-year age group as a case (55% response rate). Descriptive statistics were obtained from the cancer registry of the characteristics of cases diagnosed in a similar time period to this recruitment to demonstrate the comparability of the sample with the incident case population as described elsewhere (Newton-Bishop et al paper in submission).

Data Collection

An initial questionnaire (including a residence calendar) was completed by the participants, at home, and mailed to the interviewer, and more detailed sun exposure data were subsequently collected by telephone based upon the residence calendar as described by Armstrong (20). Comprehensive calendar data on self-reported weekday and weekend sun exposure habits throughout life were collected, as were details about latitude of residence and sun exposure habits during vacations. Age, sex, natural hair color at age 18 years,

propensity to burn, ability to tan, skin color of inside upper arm and freckling as a child using Gallagher's freckle chart (21) were recorded, based on self-report. Self reported freckling as a child showed good correlation with nurses' examined score, $p<0.0001$ for linear trend. The nurse examined mean scores were 10.0, 14.5, 24.2 and 40.4 respectively for self-reported categories none, very few, few/some and many respectively.

Cases and controls were also examined by research nurses, who recorded eye color (blue/grey, green/hazel or brown) and freckling scores for face, arms and shoulders using Gallagher's chart (21). Three nurses were trained to count nevi by JNB. Nevi ≥2 mm in diameter were counted on exposed skin: sites not examined were the genitalia and breasts in women. The counts were subdivided into 18 body sites; nevi ≥5mm in diameter and clinically atypical nevi were separately tabulated in each body site. An atypical nevus was defined as a nevus ≥5 mm in diameter, with variable pigmentation and an irregular or diffuse edge.

Genotyping

DNA was extracted from blood from consenting participants to allow detection of SNPs (19). The SNPs rs12203592 (*IRF4*, chromosome 6), rs7023329 (*MTAP*, chromosome 9) and rs2284063 (*PLA2G6*, chromosome 22) were genotyped using the Taqman genotyping assays C__31918199_10, C__29146385_10 and C___2458775_1_ respectively (Applied Biosystems, Foster City, USA). 2μl PCR reactions were performed in 384 well plates using 10ng of DNA (dried), using 0.05 μl assay mix and 1μl Universal Master Mix (Applied Biosystems, Foster City, USA) according to the manufacturer's instructions. End point reading of the genotypes was performed using an ABI 7900HT Real-time PCR system (Applied Biosystems, Foster City, USA).

The entire coding region of the *MC1R* gene was screened for variants by Sanger sequencing. The single *MC1R* exon was PCR amplified in three overlapping fragments. Each fragment was sequenced in both directions using the primers from the initial amplification. Sequencing reactions were performed as described previously (22). The sequencing results were collated and variants identified were assigned a status as ‘R’ (strong association with red hair phenotype), ‘r’ (weak association with red hair phenotype), or not associated with red hair, according to a previous widely-used classification (23).

Statistical methods

Several sun exposure measures were derived from the questionnaire data: average hours of weekday sun exposure, average hours of weekend sun exposure, average daily sun exposure, average holiday exposure, and average exposure on holidays in sunny climates (defined as at latitude below 45°). Aggregated sun exposure variables were classified into thirds based on the distribution in the control population. Data on self-reported significant sunburns (defined as causing pain for two or more days) were dichotomised as ever/never reporting sunburn, both under the age of 20 years and at or over the age of 20 years.

Distribution of whole body nevus number was fairly skewed, and log-transformation was applied to attain normality (Figure S1). Because nevi develop in the earlier years of life and then involute, estimated age-sex adjusted log-transformed nevus numbers for all participants were calculated based on regression of the log body nevus number on age and sex in the control population. Residual log body nevus number was then calculated as the log-transformed total body nevus number with the estimated age-sex adjusted value subtracted. We further classified body nevus number into quarters based on the residual log body nevus number distribution in the control population.

Linear regression models were used to estimate the effects (regression coefficients, β) of sun exposure patterns and nevus genotype on residual log body nevus number. Negative binomial regression models were used to estimate effects of sun exposure and nevus genotype on nevus number, and rate ratios (RR) and 95% confidence intervals (CI) were estimated. Modified Poisson regression with robust error variance models were used to analyze the presence of atypical nevi. Interactions between sun exposure and nevus genotype on nevus phenotype were tested by comparing a model with main effects and an interaction term with a reduced model with only main effects, using the likelihood ratio test. Unconditional logistic regression models were used to examine the effects of nevus phenotype and nevus genotype on melanoma risk, and odds ratios (OR) and 95% CI were estimated. Sums-of-squares R^2 $\left(R^2_{ss}\right)$, defined as $R^2_{ss}=1-\frac{SSE}{SST}=1-\frac{\sum_i (y_i-\widehat{p_i})^2}{\sum_i (y_i-\overline{p})^2}$, where $y_i=0$ for control and 1 for case, $\hat{p}_i$ is the estimated probability of being affected, based on the fitted model, and $\overline{p}=\sum_i y_i/N$, was used to assess the proportion of variation explained by covariates in the logistic regression models (24). These analyses were carried out using the GLM, GENMOD and LOGISTIC procedures in SAS version 9.1 for PC (Copyright, SAS Institute Inc. Cary, NC, USA).

Results

As nevus number is believed to be determined, at least in part, by sun exposure, we first looked at the relationship between counts of all nevi and clinically atypical nevi, and different patterns of sun exposure (Table 1). Correlations with sun exposure were determined for cases and controls separately and then for all cases and controls together. In Table 1 it can be seen that there was a strong positive correlation between total nevus number and holiday sun exposure. For example cases in the highest tertile of holiday sun exposure below 45° had a median nevus count of 56 compared with 30 for those in the lowest tertile, and a similar increase was seen in controls. There was no convincing relationship between either average daily exposure or sunburn and nevus number.

Holiday sun exposure was also associated with an increased number of atypical nevi, particularly for very sunny holidays at below 45° latitude (presence of at least one atypical nevus 58% more likely respectively for those in the middle and highest tertiles of exposure compared with the lowest). As total nevus number and number of atypical nevi have been shown in other studies to be highly correlated (25), we looked at the relationship between sun exposure and atypical nevi corrected for total nevus number. The effect of holiday sun exposure on atypical nevi persisted only for holidays at less than 45° latitude (p=0.01).

We examined the relationship between sun exposure and nevi ≥5mm in diameter, and the data support a relationship between holiday sun exposure and number of large nevi, although the effect was only seen for holidays at latitudes higher than 45°, individuals in the highest tertile of exposure having on average 45% more large nevi than those in the lowest tertile of holiday sun exposure above 45° (rate ratio 1.45, p=0.0007 for trend, data not shown). We investigated the relationship between sun exposure in continuously exposed body sites such as the arms, and in intermittently exposed sites such as the trunk (Table S1). The relationship between holiday exposure and nevus number was more marked for intermittently exposed sites (p<0.0001 for all categories of average holiday exposure, regression coefficient β = 0.32 on log scale corresponding to a 38% increase in nevus number comparing highest to lowest tertile of exposure, than for continuously exposed sites (p=0.0005, β = 0.20, 22% increase). There was weak evidence that higher average weekend

sun exposure in warmer months was associated with fewer nevi in continuously exposed sites (p=0.05, β = -0.11, 10% decrease).

We then confirmed the relationship between inheritance of SNPs on chromosomes 9 (rs7023329), 6 (re12203592), and 22 (rs2284063) and nevus phenotype (Table 2). The A allele at the SNP on chromosome 9 was associated with reduced total nevus number (p value in a test for trend 0.04; median nevus count of 41 in cases with the AA genotype compared with 43 in those with GG genotype and a similar reduction to 15 from 17 in controls), and reduced number of large nevi (≥5mm), (p=0.03 for trend, number of large nevi in those with AA genotype 77% of that for the GG genotype). There was no significant protective effect for clinically atypical nevi, but the regression coefficients were of a similar order of magnitude as for large nevi. The association between this SNP and fewer nevi was similar for 1 and for 2 A alleles, suggesting a dominant effect. The chromosome 9 SNP showed weak evidence of an interaction between sunburn and sun exposure and genotype (Table S2) on nevus phenotype, represented graphically in Figure 1. The effect of sunburn at or over the age of 20 years for heterozygotes was a 14% increase in total body nevus number (p=0.09) and a 33% increase in large nevi (p=0.03). The effect of sunburn for GG homozygotes was a 31% increase in total body nevus number (p=0.01) and a 40% increase in large nevi (p=0.07). There was no effect of sunburn on nevi in individuals with the AA genotype (p=0.69 and 0.41 for total body nevi and number of large normal nevi respectively).

The T allele for the chromosome 6 *IRF4* SNP showed some evidence of association with fewer large nevi (rate ratio 0.52 for TT versus CC genotype, p=0.01 for trend) (Table 2) and less significantly with reduced risk of atypical nevi but not total nevus count. The A allele at the SNP on chromosome 22 was associated with both a reduced total number of nevi (median of 39 in cases with the AA genotype compared with 45 with the GG genotype and a less striking difference in controls, overall p=0.002 for trend) and a reduced number of large nevi (rate ratio 0.73 for AA versus GG, p=0.002 for trend), but there was no association with clinically atypical nevi. There was some evidence of an interaction between this SNP and sunburn under the age of 20 years, on the number of large and atypical nevi (Table S2). SNPs were associated similarly with nevus number in continuously exposed and intermittently exposed sites (Table S3).

The major interest in nevus genes is their effect on melanoma risk, and we therefore looked at the relationship between nevus phenotype and genotype (Table 3) and risk. In these analyses we corrected for other variables in the table and also for age, sex and sun sensitivity phenotypes such as hair color and freckling. The analysis confirmed the strong relationship between nevus number and melanoma risk, with a crude odds ratio (OR) for melanoma of 10.02 (95% CI 6.91-14.52) comparing the top quartile with the lowest quartile of nevus count. The relationship persisted when adjusted for number of atypical nevi and genotype, OR 7.47 (95% CI 5.01-11.14), and sun sensitivity phenotypes, OR 11.66 (95% CI 7.78-17.48). Inheritance of the rarer allele at the SNPs on chromosomes 6, 9 and 22 was associated with a reduced risk of melanoma, and these effects persisted when adjusted for other SNPs and the nevus phenotype (ORs of 0.83 (95% CI 0.67-1.03), 0.85 (95% CI 0.70-1.02) and 0.86 (95% CI 0.71-1.04) per allele, respectively, for SNPs on chromosomes 6, 9, and 22, assuming an additive mode of inheritance).

As sun sensitivity is associated with an increased risk of melanoma, and inheritance of variants in the *MC1R* gene is the major determinant of sun sensitivity (23, 26), we investigated the relationship between the three nevus SNPs and melanoma risk stratified by the presence or absence of 1 or more ‘R’ *MC1R* variants, that is variants known to be strongly associated with the sun sensitive phenotype (27) (Table S4), but there was no evidence of a differential effect. In Table 4, we report the association between nevus

number, number of atypical nevi and inheritance of the nevus SNPs and melanoma risk at different body sites. It is seen that nevus number, number of atypical nevi and inheritance of the chromosome 6 and 9 nevus SNPs were more strongly associated with risk of truncal melanoma than melanoma at other common sites. Nevus number was however also strongly associated with risk of melanoma on the limbs, and notably also with melanoma at other sites, including rare sites (pooled acral lentiginous melanoma, genital melanoma and melanoma arising in the ear nose and throat).

Finally, in Table 5 we report the effect of nevus number and inheritance of nevus SNPs on melanoma arising at different ages, since nevus phenotype changes with age. The risk associated with increased nevus number was similar under the age of 50 years and in older individuals. The risk associated with atypical nevi appeared greater in cases aged over 50 ($p=0.001$) than in those under the age of 50 ($p=0.11$), but the difference was not statistically significant. Similarly the risk associated with inheritance of the nevus SNP on chromosome 22 appeared to differ by age, so that the A allele was more strongly protective for those under the age of 50 years ($p=0.003$) than those aged over 50 ($p=0.15$), but the difference was not statistically significant. The risk associated with the chromosome 6 and 9 SNPs was similar in the two age groups.

We estimated the proportion of the variation in melanoma risk explained by nevus genotype, nevus phenotype and pigmentation phenotype (Table S5). The nevus SNPs, considered independently, each explained less than 1% of the variation, and by combining all SNPs, only 2% was explained. The nevus phenotype however explained 19% and pigmentation phenotypes 4% of the variation. The highest proportion of risk explained was 23%, when the nevus SNPs and nevus and pigmentation phenotypes were combined.

Discussion

As melanoma continues to increase in incidence in many parts of the world, it remains crucial to identify risk factors for melanoma and to understand the effects of both environmental exposures and susceptibility genes on risk. We report here on the effect of nevus-related SNPs associated with melanoma risk in a large case-control sample recruited in the UK. In this area of the world the number of melanoma cases has increased (28), so that it has become the commonest cancer in young British adults (Office of National Statistics Data).

Pooled data analyses of case-control studies have confirmed that the major environmental exposure associated with melanoma risk is holiday sun exposure (7), particularly in temperate climes and that the strongest phenotypic risk factor is the presence of increased number of nevi (9). Previous twin studies in adolescents have suggested that holiday sun exposure is predictive of an increased number of nevi (17), and a number of studies in healthy individuals have shown a relationship between sun exposure and nevus number (16, 29-31). In this study, we have provided further evidence that holiday sun exposure, rather than total (daily) sun exposure, is the major determinant of nevus number in adults, and that holiday sun exposure is also correlated with larger nevi (indicative of more proliferative melanocytes) and (to a lesser extent) clinically atypical nevi. The data reported here do support the view that behavior in the sun is associated with melanocyte proliferation, at least in those with a susceptible phenotype.

It is of interest that we found some weak evidence that nevus number on continuously sun-exposed sites such as the arms was lower ($p=0.05$) in those with higher levels of weekend sun exposure in warmer months in this temperate climate. This is consistent with our recent observation that higher levels of weekend sun exposure were protective for melanoma in the

UK (Newton-Bishop et al in submission). That nevus number appeared to be negatively correlated with greater weekend sun exposure in a temperate climate, might suggest that although sunny holidays and sunburn increased nevus number, regular (moderate) sun exposure may also be in some way related to inhibition of melanocyte proliferation. We suggest that this is consistent with the observation that larger, clinically atypical nevi are most numerous on intermittently exposed skin, such as the back (30), even though the number of small nevi on the arms may be higher (31). Thus the data support the view that the relationship between sun exposure and melanoma risk is complex.

In previous case-control (32) studies, increased nevus number and actinic keratoses (as a marker of chronic sun exposure in the fair skinned) were both reported to be predictive of melanoma risk, but the phenotypic markers were negatively correlated. That is, that increased number of nevi and actinic keratoses were both risk factors but they occurred in different people indicating the probability of two different “routes” to melanoma. Other observations added to the suggestion that there was more than one “route” to melanoma (33), and this has been substantiated more recently by the identification of biological differences between tumors which have developed on intermittently sun-exposed body sites and those on continuously exposed sites (34). In this study we have shown that increased nevus number is related to holiday sun exposure, and although nevus number was predictive of melanoma risk overall, the strongest relationship was with melanoma on the trunk (Table 4), which is the most intermittently sun-exposed body site. Our data are therefore consistent with this “two route hypothesis”, and indeed with other studies including a pooled data analysis of case-control study data in women, recently reported (35, 36) which was carried out to test the hypothesis. However it is of note that in our study increased nevus number was associated with melanoma risk for tumors at all body sites, suggesting that patients with the Atypical Mole Syndrome are at increased risk of melanoma even in rare sites such as acral lentiginous melanoma, and that the relationship between tumor site and different biological pathways to melanoma is likely complex.

We have confirmed that SNPs in *IRF4* on chromosome 6 and additional SNPs on chromosomes 9 and 22 influence the nevus phenotype, and are associated with melanoma risk. Of these SNPs, those on chromosomes 9 and 22 were most strongly predictive of nevus number. The relationship between the *IRF4* SNP and nevus number is reported to be complex and dependent on age (Duffy et al, in press), which may explain the lack of association with total nevus count in these data.

We showed that the proportion of melanoma risk explained by these SNP genotypes is small compared to what is explained by nevus phenotype. The evidence is strong from twin studies that nevus number is primarily under genetic control (13, 15, 16); the implication is therefore that there are significant numbers of nevus (hypothesized to be melanoma susceptibility) genes yet to be identified.

In summary, these data confirm that increased nevus number and bigger nevi (indicative of a more highly proliferative melanocyte population) are associated with increased melanoma risk. That association is particularly strong for melanoma on the trunk and limbs, but is detected for all body sites, even in non-sun exposed skin. Thus, although our data support the “two route hypothesis to melanoma”, they also support the view that melanomas related to the inheritance of nevus genes occur all over the skin. The three SNPs on chromosomes 6, 9 and 22 are related both to nevus number and to melanoma risk, but a large proportion of the nevus phenotype remains unexplained. Further exploration of the genetic determinants of nevi using both genome wide association and candidate gene studies will be needed to better understand the hereditary variation which determines melanocyte responses to sun exposure and therefore melanoma risk.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

The collection of samples in the Melanoma Cohort Study was funded by Cancer Research UK (project grant C8216/A6129 and programme award C588/A4994) and by the NIH (R01 CA83115). Recruitment was facilitated by the UK National Cancer Research Network. Patricia Mack, and Kate Gamble collected data for the studies. Paul King carried out data entry. Dr Amy Downing of NYCRIS provided cancer registry data.

The following recruited patients to the studies: Mr J Ausobsky, Yorkshire Clinic; Dr A Carmichael, Mr M Coady, Dr S Shehade, Mr H Siddiqui, Mr K Allison, Mr K Erdinger, Mr Ramanathan, Mr Toby Muir, James Cook University Hospital; Dr A S Highet, Mr K R Mannur, Mr M Telfer, Dr K Thomson, Mr G Miller, Mr J M Hayward, Mr J Taylor, Mr A Coatesworth, Dr A E Myatt, Dr J Schofield, Dr Callum Lyon, York District Hospital & Scarborough Hospital; Dr Alison Layton, Harrogate District Hospital; Dr Anthony Maraveyas, Dr S Walton, Dr N Alexander, Mr Alistair Platt, Mr N B Hart, Mr P M O'Hare, Mr P Stanley, Mr M Riaz, Mr Ramakrishnan, Castle Hill Hospital and Princess Royal Hospitals Hull; Dr D Seukeran, Friarage Hospital; Dr Bruce Pollock, Dr E D A Potts, Dr S Clark, Dr S MacDonald Hull, Mr L Le Roux Fourie, Miss O M B Austin, Mr S Southern, Mr O M Fenton, Mr S Majumder, Mr A R Phipps, Pinderfields Hospital; Dr D Cowan, Dr H Hempel, Dr J Holder, Dr M Cheesbrough, Dr H Galvin, Mr D Sutton, Huddersfield Royal Infirmary, Dr I Barbar, Calderdale Royal Hospital, Dr J A A Langtry, Dr S Natarajan, Dr Verlangi, Sunderland Royal Hospital; Dr Neil Cox, Cumberland Infirmary; Mr R Debono, University Hospital North Durham; Mr P Baguley, Middlesborough General Hospital; Mr R B Berry, Mr M Erdmann, Mr N McLean, Mr S Rao, University Hospital North Durham; Mr S L Knight, Mr C Fenn, Dr M Marples, Mr Mark Liddington, Mr Simon Kay, Mr Howard Peach, Mr Andrew Batchelor, Dr Michelle Cronk, Dr R Sheehan-Dare, Dr Mark Goodfield, Dr Poulam Patel, Dr Victoria Goulden, Dr Alison Humphreys, Mr K Horgan, Mr Chips Browning, Dr Graeme Stables, Dr Sabine Sommer, Dr Caroline Wilson and Dr S M Wilkinson, Leeds Teaching Hospitals Trust; Mr Sugden, University Hospital Hartlepool, Dr Andrew Wright, Mr Al Ghazal, Mr S F Worrall, Mr R M Antrum, Mr Ivan Foo, Mr D Watt, Dr K London, Dr D J Barker, Prof D T Sharpe, Mr M Timmons, Bradford Royal Infirmary and Airedale Hospital; Dr G P Ford, Dr G Taylor, Dr M Shah, Dewsbury and District Hospital. These pathologists also assisted: Dr A Clarke, York District Hospital; Dr A Gledhill, Harrogate; Dr A Roy, Hull; Dr Sara Edwards, Dr Andrew Boon and Dr Will Merchant, Leeds Teaching Hospitals Trust; Dr Srikantiah Nagara, and Dr Hugh Cochrane, Sunderland Hospital; Dr Paul Barrett, University Hospital North Durham; Dr David Henderson, Friarage Hospital; Airedale General; Dr John J O'Dowd and Dr Phillip Batman, Bradford Royal Infirmary; Dr Patricia W Gudgeon and Dr U Raja and Dr I W Claire MacDonald, Dewsbury/Pontefract; Dr George D H Thomas, Huddersfield Royal Infirmary; Dr Alan Padwell, Calderdale.

References

1. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: III. Family history, actinic damage and phenotypic factors. Eur J Cancer. 2005; 41:2040–59. [PubMed: 16125929]
2. Valverde P, Healy E, Jackson I, Rees J, Thody A. Variants of the melanocyte stimulating hormone receptor gene are associated with red hair and fair skin in humans. Nature Genetics. 1995; 11:328–30. [PubMed: 7581459]
3. Valverde P, Healy E, Sikkink S, et al. The Asp84Glu variant of the melanocortin 1 receptor (MC1R) is associated with melanoma. Human Molec Genet. 1996; 5:1663–6. [PubMed: 8894704]
4. Brown KM, Macgregor S, Montgomery GW, et al. Common sequence variants on 20q11.22 confer melanoma susceptibility. Nat Genet. 2008; 40:838–40. [PubMed: 18488026]
5. Gudbjartsson DF, Sulem P, Stacey SN, et al. ASIP and TYR pigmentation variants associate with cutaneous melanoma and basal cell carcinoma. Nat Genet. 2008
6. Bishop DT, Demenais F, Iles MM, et al. Genome-wide association study identifies three loci associated with melanoma risk. Nat Genet. 2009; 41:920–5. [PubMed: 19578364]
7. Chang YM, Barrett JH, Bishop DT, et al. Sun exposure and melanoma risk at different latitudes: a pooled analysis of 5700 cases and 7216 controls. Int J Epidemiol. 2009; 38:814–30. [PubMed: 19359257]
8. Gandini S, Sera F, Cattaruzza MS, et al. Meta-analysis of risk factors for cutaneous melanoma: I. Common and atypical naevi. Eur J Cancer. 2005; 41:28–44. [PubMed: 15617989]

9. Chang YM, Newton-Bishop JA, Bishop DT, et al. A pooled analysis of melanocytic nevus phenotype and the risk of cutaneous melanoma at different latitudes. Int J Cancer. 2009; 124:420–8. [PubMed: 18792098]
10. Pollock PM, Harper UL, Hansen KS, et al. High frequency of BRAF mutations in nevi. Nat Genet. 2003; 33:19–20. [PubMed: 12447372]
11. Omholt K, Platz A, Kanter L, Ringborg U, Hansson J. NRAS and BRAF mutations arise early during melanoma pathogenesis and are preserved throughout tumor progression. Clin Cancer Res. 2003; 9:6483–8. [PubMed: 14695152]
12. Gray-Schopfer VC, Cheong SC, Chong H, et al. Cellular senescence in naevi and immortalisation in melanoma: a role for p16? Br J Cancer. 2006; 95:496–505. [PubMed: 16880792]
13. Wachsmuth RC, Gaut RM, Barrett JH, et al. Heritability and gene-environment interactions for melanocytic nevus density examined in a U.K. adolescent twin study. J Invest Dermatol. 2001; 117:348–52. [PubMed: 11511314]
14. Easton D, Cox G, Macdonald A, Ponder B. Genetic susceptibility to naevi- a twin study. Br J Cancer. 1991; 64:1164–7. [PubMed: 1764382]
15. Zhu G, Duffy DL, Eldridge A, et al. A major quantitative-trait locus for mole density is linked to the familial melanoma gene CDKN2A: a maximum-likelihood combined linkage and association analysis in twins and their sibs. Am J Hum Genet. 1999; 65:483–92. [PubMed: 10417291]
16. Bataille V, Snieder H, MacGregor AJ, Sasieni P, Spector TD. Genetics of risk factors for melanoma: an adult twin study of nevi and freckles. J Natl Cancer Inst. 2000; 92:457–63. [PubMed: 10716963]
17. Wachsmuth RC, Turner F, Barrett JH, et al. The Effect of Sun Exposure in Determining Nevus Density in UK Adolescent Twins. J Invest Dermatol. 2005; 124:56–62. [PubMed: 15654953]
18. Falchi M, Bataille V, Hayward NK, et al. Genome-wide association study identifies variants at 9p21 and 22q13 associated with development of cutaneous nevi. Nat Genet. 2009; 41:915–9. [PubMed: 19578365]
19. Randerson-Moor JA, Taylor JC, Elliott F, et al. Vitamin D receptor gene polymorphisms, serum 25-hydroxyvitamin D levels, and melanoma: UK case-control comparisons and a meta-analysis of published VDR data. Eur J Cancer. 2009; 45:3271–81. [PubMed: 19615888]
20. Karipidis KK, Benke G, Sim MR, et al. Non-Hodgkin lymphoma and occupational radiation exposure assessed using local data. Occup Med (Lond). 2009; 59:437–9. [PubMed: 19578076]
21. Lee TK, Rivers JK, Gallagher RP. Site-specific protective effect of broad-spectrum sunscreen on nevus development among white schoolchildren in a randomized trial. J Am Acad Dermatol. 2005; 52:786–92. [PubMed: 15858467]
22. Harland M, Goldstein AM, Kukalizch K, et al. A comparison of CDKN2A mutation detection within the Melanoma Genetics Consortium (GenoMEL). Eur J Cancer. 2008; 44:1269–74. [PubMed: 18394881]
23. Raimondi S, Sera F, Gandini S, et al. MC1R variants, melanoma and red hair color phenotype: a meta-analysis. Int J Cancer. 2008; 122:2753–60. [PubMed: 18366057]
24. Mittlbock M, Schemper M. Explained variation for logistic regression. Stat Med. 1996; 15:1987–97. [PubMed: 8896134]
25. Bataille V, Newton Bishop JA, Sasieni P, et al. Risk of cutaneous melanoma in relation to the numbers, types and sites of naevi: a case-control study. Br J Cancer. 1996; 73:1605–11. [PubMed: 8664138]
26. Sturm RA. Skin colour and skin cancer - MC1R, the genetic link. Melanoma Res. 2002; 12:405–16. [PubMed: 12394181]
27. Healy E. Melanocortin 1 receptor variants, pigmentation, and skin cancer susceptibility. Photodermatol Photoimmunol Photomed. 2004; 20:283–8. [PubMed: 15533235]
28. Downing A, Newton-Bishop JA, Forman D. Recent trends in cutaneous malignant melanoma in the Yorkshire region of England; incidence, mortality and survival in relation to stage of disease, 1993-2003. Br J Cancer. 2006; 95:91–5. [PubMed: 16755289]
29. Dulon M, Weichenthal M, Blettner M, et al. Sun exposure and number of nevi in 5- to 6-year-old European children. J Clin Epidemiol. 2002; 55:1075–81. [PubMed: 12507670]

30. Silva Idos S, Higgins CD, Abramsky T, et al. Overseas sun exposure, nevus counts, and premature skin aging in young English women: a population-based survey. J Invest Dermatol. 2009; 129:50–9. [PubMed: 18615111]

31. Carli P, Naldi L, Lovati S, La Vecchia C. The density of melanocytic nevi correlates with constitutional variables and history of sunburns: a prevalence study among Italian schoolchildren. Int J Cancer. 2002; 101:375–9. [PubMed: 12209963]

32. Bataille V, Sasieni P, Grulich A, et al. Solar keratoses: a risk factor for melanoma but negative association with melanocytic naevi. Int J Cancer. 1998; 78:8–12. [PubMed: 9724086]

33. Whiteman DC, Watt P, Purdie DM, Hughes MC, Hayward NK, Green AC. Melanocytic nevi, solar keratoses, and divergent pathways to cutaneous melanoma. J Natl Cancer Inst. 2003; 95:806–12. [PubMed: 12783935]

34. Curtin JA, Fridlyand J, Kageshita T, et al. Distinct sets of genetic alterations in melanoma. N Engl J Med. 2005; 353:2135–47. [PubMed: 16291983]

35. Olsen CM, Zens MS, Stukel TA, et al. Nevus density and melanoma risk in women: a pooled analysis to test the divergent pathway hypothesis. Int J Cancer. 2009; 124:937–44. [PubMed: 19035450]

36. Siskind V, Whiteman DC, Aitken JF, Martin NG, Green AC. An analysis of risk factors for cutaneous melanoma by anatomical site (Australia). Cancer Causes Control. 2005; 16:193–9. [PubMed: 15947871]

Abbreviations

SNP	single nucleotide polymorphism
OR	odds ratio

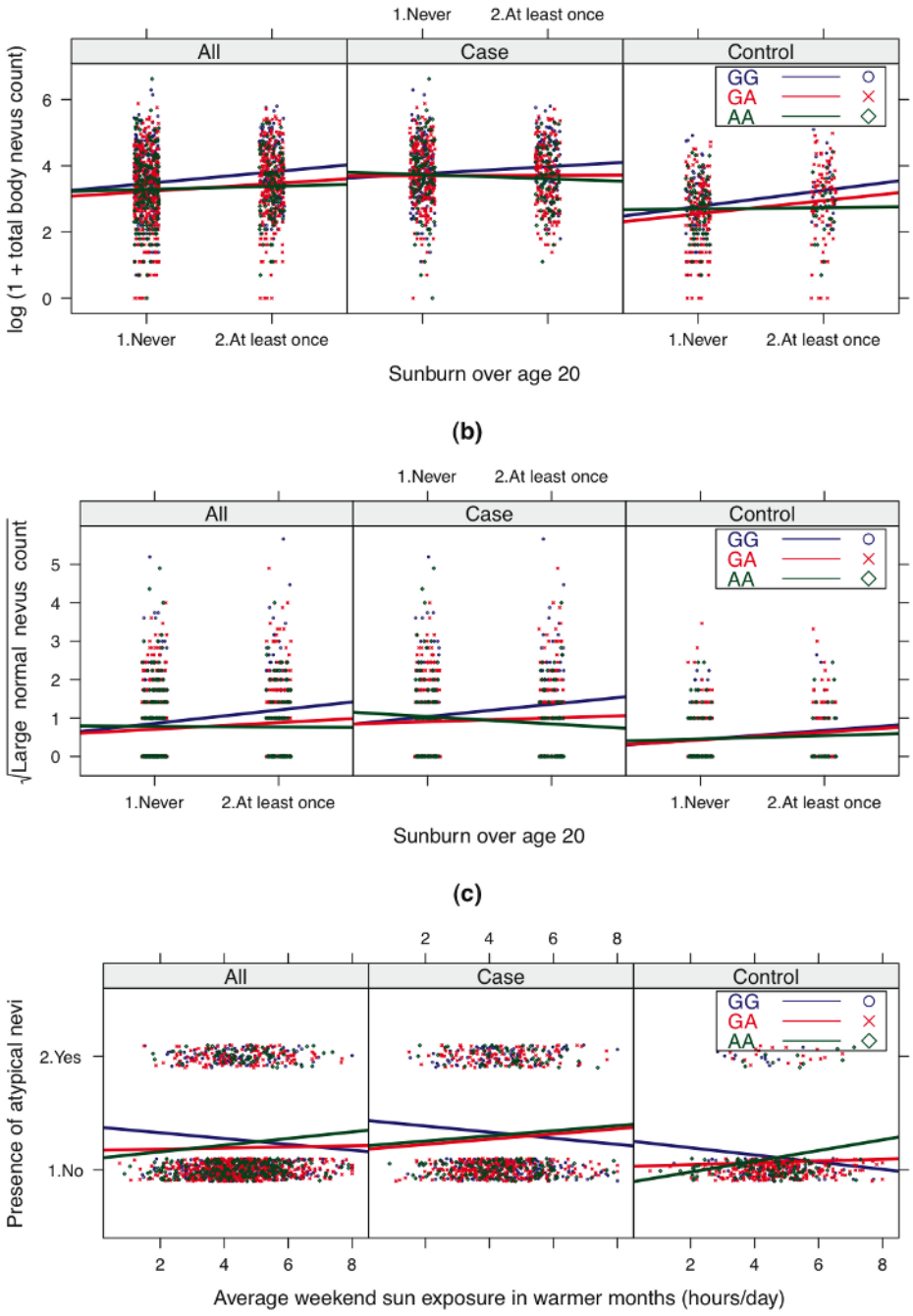

Figure 1.
Interaction between sun exposure and rs7023329_chr9 variant on (a) total body nevus number, (b) large normal nevi, and (c) presence of atypical nevi.

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Table 1

Association between different patterns of sun exposure and total body nevus number, number of large normal nevi and presence of atypical nevi adjusted for case-control status.

Sun exposure	N (%)	Total body nevus number*				Number of large nevi^		Presence of atypical nevi§		
		Controls Median (Q1, Q3)	Cases Median (Q1, Q3)	β (95% CI)	P trend	Rate Ratio (95% CI)	P trend	Risk Ratio (95% CI)	P trend	P trend&
Sunburn under age 20 years										
No	902 (68)	15 (7,29)	42 (21, 76)	-		-		-		
Yes	423 (32)	18 (10, 37)	42 (22, 98)	0.03 (-0.08, 0.15)	0.55	1.09 (0.90, 1.32)	0.37	1.12 (0.92, 1.38)	0.25	0.20
Sunburn at/over age 20 years										
No	802 (61)	14 (6, 29)	42 (21, 83)	-		-		-		
Yes	504 (39)	19 (10, 36)	43 (20, 89)	0.10 (-0.01, 0.21)	0.06	1.18 (0.98, 1.41)	0.08	1.07 (0.87, 1.30)	0.54	0.86
Average weekday exposure at cooler months (hours/day)										
≤0.9	463 (34)	15 (7, 32)	42 (23, 79)	-		-		-		
0.9-1.5	463 (34)	17 (8, 29)	43 (20, 85)	0.01 (-0.12, 0.13)		1.09 (0.88, 1.34)		0.83 (0.65, 1.04)		
>1.5	440 (32)	15 (6, 29)	42 (22, 89)	0.01 (-0.12, 0.13)	0.91	1.15 (0.92, 1.43)	0.22	0.96 (0.76, 1.20)	0.68	0.84
Average weekday exposure at warmer months (hours/day)										
≤1.4	489 (36)	15 (8, 28)	42 (23, 84)	-		-		-		
1.4-2.1	415 (30)	17 (7, 32)	42 (21, 81)	0.03 (-0.10, 0.15)		0.97 (0.78, 1.21)		0.78 (0.61, 0.99)		
>2.1	466 (34)	15 (6, 29)	43 (20, 89)	-0.01 (-0.13, 0.11)	0.87	1.21 (0.98, 1.49)	0.07	0.86 (0.69, 1.07)	0.16	0.23
Average weekend exposure at cooler months (hours/day)										
≤2.5	498 (36)	14 (7, 30)	44 (23, 78)	-		-		-		
2.5-3.5	466 (34)	17 (8, 34)	41 (20, 85)	0.04 (-0.08, 0.16)		1.18 (0.96, 1.45)		0.90 (0.71, 1.12)		
>3.5	416 (30)	15 (6, 28)	44 (20, 94)	-0.03 (-0.15, 0.10)	0.71	1.01 (0.81, 1.26)	0.84	0.94 (0.75, 1.19)	0.58	0.82
Average weekend exposure at warmer months (hours/day)										
≤4.0	538 (39)	14 (7, 27)	42 (21, 78)	-		-		-		
4.0-5.0	424 (31)	15 (6, 31)	46 (23, 94)	0.03 (-0.09, 0.15)		1.07 (0.87, 1.32)		1.15 (0.92, 1.45)		
>5.0	417 (30)	18 (7, 36)	41 (19, 87)	-0.04 (-0.17, 0.08)	0.52	0.98 (0.80, 1.22)	0.94	1.15 (0.91, 1.45)	0.21	0.10
Average daily exposure (hours/day)										
≤1.9	492 (36)	15 (7, 29)	44 (24, 80)	-		-		-		
1.9-2.5	419 (31)	17 (8, 34)	40 (21, 88)	-0.01 (-0.13, 0.12)		0.94 (0.76, 1.17)		0.91 (0.72, 1.15)		
>2.5	443 (33)	15 (6, 29)	43 (20, 89)	-0.06 (-0.18, 0.07)	0.38	1.16 (0.94, 1.43)	0.18	0.91 (0.72, 1.14)	0.39	0.68

Sun exposure	N (%)	Total body nevus number*				Number of large nevi^		Presence of atypical nevi§		
		Controls Median (Q1, Q3)	Cases Median (Q1, Q3)	β (95% CI)	P trend	Rate Ratio (95% CI)	P trend	Risk Ratio (95% CI)	P trend	P trend&
Average holiday exposure (hours/year)										
≤46.5	493 (36)	14 (5, 27)	33 (19, 59)	-		-		-		
46.5-71.5	411 (30)	15 (7, 31)	48 (25, 93)	0.23 (0.10, 0.35)		1.29 (1.03, 1.60)		1.45 (1.14, 1.84)		
>71.5	467 (34)	17 (10, 34)	51 (23, 97)	0.29 (0.17, 0.41)	<0.0001	1.38 (1.12, 1.71)	0.002	1.24 (0.97, 1.58)	0.08	0.38
Average holiday exposure (10am-2pm) (hours/year)										
≤23.3	502 (37)	14 (5, 26)	34 (19, 63)	-		-		-		
23.3-38.0	435 (32)	15 (7, 31)	50 (26, 96)	0.28 (0.16, 0.40)		1.15 (0.93, 1.43)		1.15 (0.91, 1.45)		
>38.0	429 (31)	17 (10, 36)	46 (21, 93)	0.28 (0.16, 0.40)	<0.0001	1.33 (1.07, 1.64)	0.009	1.08 (0.85, 1.37)	0.48	0.85
Average holiday exposure below 45°N (hours/year)										
≤6.5	456 (33)	11 (4, 23)	30 (14, 61)	-		-		-		
6.5-26.7	446 (33)	15 (8, 34)	42 (23, 87)	0.19 (0.07, 0.32)		1.03 (0.83, 1.28)		1.58 (1.21, 2.04)		
>26.7	469 (34)	20 (10, 36)	56 (27, 101)	0.31 (0.19, 0.43)	<0.0001	1.06 (0.85, 1.31)	0.61	1.57 (1.21, 2.02)	0.0006	0.01
Average holiday exposure below 45°N (10am-2pm) (hours/year)										
≤3.3	471 (35)	12 (4, 23)	32 (17, 61)	-		-		-		
3.3-13.0	434 (32)	15 (8, 32)	43 (23, 94)	0.20 (0.07, 0.32)		1.03 (0.83, 1.28)		1.58 (1.22, 2.03)		
>13.0	461 (34)	20 (10, 35)	52 (26, 96)	0.26 (0.13, 0.38)	<0.0001	0.98 (0.79, 1.22)	0.88	1.51 (1.17, 1.94)	0.001	0.02

*Median total body nevus number (first quartile, third quartile) for controls and cases are presented separately but subsequent analyses are for the total sample adjusted for case-control status. Age-sex corrected log-transformed body nevus number was used to test for association, and the linear regression coefficient (β) and its 95% confidence interval are presented;

^Analyses were carried out using Negative Binomial regression models and rate ratios are presented;

§Modified Poisson regression models with robust error variance were used to analyze presence of atypical nevi and risk ratios are presented;

&log-transformed body nevus number was further adjusted for in the trend test.

Table 2

Association between nevus genotype and total body nevus number, large normal nevus number and presence of atypical nevi adjusted for case-control status.

Nevus genotype	N (%)	Total body nevus number*				Number of large nevi^		Presence of atypical nevi§	
		Controls Median (Q1, Q3)	Cases Median (Q1, Q3)	β (95% CI)	P trend	Rate Ratio (95% CI)	P trend	Risk Ratio (95% CI)	P trend
rs12203592_chr6									
CC	872 (62%)	16 (8, 29)	41 (21, 77)	-		-		-	
CT	463 (33%)	15 (6, 30)	44 (21, 94)	0.04 (-0.07, 0.15)		0.88 (0.73, 1.06)		0.86 (0.69, 1.06)	
TT	64 (5%)	14 (8, 24)	33 (14, 62)	-0.27 (-0.51, -0.03)	0.43	0.52 (0.33, 0.83)	0.01	0.67 (0.38, 1.18)	0.05
rs7023329_chr9									
GG	343 (26%)	17 (8, 32)	43 (21, 93)	-		-		-	
GA	713 (53%)	15 (7, 29)	41 (20, 78)	-0.15 (-0.28, -0.02)		0.79 (0.64, 0.98)		0.82 (0.66, 1.03)	
AA	289 (21%)	15 (7, 24)	41 (20, 77)	-0.15 (-0.30, -0.00)	0.04	0.77 (0.59, 0.99)	0.03	0.94 (0.71, 1.23)	0.54
rs2284063_chr22									
GG	611 (44%)	17 (8, 35)	45 (21, 89)	-		-		-	
GA	620 (45%)	15 (7, 27)	40 (20, 77)	-0.12 (-0.23, -0.02)		0.75 (0.62, 0.90)		0.99 (0.81, 1.21)	
AA	152 (11%)	13 (6, 28)	39 (20, 77)	-0.24 (-0.41, -0.07)	0.002	0.73 (0.54, 0.98)	0.002	1.05 (0.76, 1.46)	0.87

*Median total body nevus number (first quartile, third quartile) for controls and cases are presented separately, but subsequent analyses are for the total sample adjusted for case-control status; age-sex corrected log-transformed body nevus number was used to test for association, and linear regression coefficient (β) and its 95% confidence interval are presented;

^Analyses were carried out using Negative Binomial regression models and rate ratios are presented;

§Modified Poisson regression models with robust error variance were used to analyze presence of atypical nevi and risk ratios are presented.

Table 3

Association between nevus phenotype and nevus genotype and risk of melanoma

Risk factor	Control	Case	Univariate OR (95% CI)	Multivariable OR1 (95% CI)*	Adjusted OR2 (95% CI)^
Residual log body nevus number quartile					
Q1	126 (25)	55 (6)	-	-	-
Q2	126 (25)	127 (14)	**2.31 (1.55, 3.45)**	**1.98 (1.29, 3.03)**	**2.64 (1.71, 4.08)**
Q3	126 (25)	205 (22)	**3.73 (2.53, 5.49)**	**3.12 (2.07, 4.70)**	**4.32 (2.84, 6.59)**
Q4	126 (25)	551 (59)	**10.02 (6.91, 14.52)**	**7.47 (5.01, 11.14)**	**11.66 (7.78, 17.48)**
Atypical nevi					
0	462 (92)	657 (70)	-	-	-
1+	42 (8)	281 (30)	**4.70 (3.33, 6.64)**	**2.80 (1.91, 4.10)**	**3.95 (2.75, 5.66)**
rs12203592_chr6					
CC	279 (57)	598 (65)	-	-	-
CT	185 (38)	279 (30)	**0.70 (0.56, 0.89)**	**0.66 (0.51, 0.86)**	**0.67 (0.52, 0.87)**
TT	22 (5)	42 (5)	0.89 (0.52, 1.52)	1.04 (0.57, 1.91)	0.80 (0.44, 1.44)
Linear trend			**0.80 (0.66, 0.96)**	0.83 (0.67, 1.03)	**0.76 (0.61, 0.94)**
rs7023329_chr9					
GG	94 (20)	250 (29)	-	-	-
GA	274 (58)	441 (50)	**0.61 (0.46, 0.80)**	**0.65 (0.48, 0.88)**	**0.58 (0.43, 0.77)**
AA	107 (23)	184 (21)	**0.65 (0.46, 0.90)**	0.70 (0.48, 1.01)	**0.63 (0.45, 0.90)**
Linear trend			**0.80 (0.68, 0.95)**	0.85 (0.70, 1.02)	**0.79 (0.67, 0.94)**
rs2284063_chr22					
GG	193 (40)	421 (47	-	-	-
GA	226 (47)	396 (44)	0.80 (0.63, 1.02)	0.86 (0.66, 1.13)	0.82 (0.64, 1.06)
AA	66 (14)	87 (10)	**0.60 (0.42, 0.87)**	0.72 (0.48, 1.09)	**0.61 (0.42, 0.89)**
Linear trend			**0.79 (0.67, 0.93)**	0.86 (0.71, 1.04)	**0.79 (0.67, 0.94)**
Total number of variants (T allele for rs12203592_chr6, and A allele for rs7023329_chr9 and rs2284063_chr22)					
0/1	122 (26)	303 (35)	-	-	-
2	154 (33)	306 (36)	0.80 (0.60, 1.07)	0.81 (0.59, 1.11)	0.75 (0.56, 1.02)
3	133 (29)	186 (22)	**0.56 (0.42, 0.77)**	**0.67 (0.48, 0.95)**	**0.55 (0.40, 0.76)**
4+	57 (12)	60 (7)	**0.42 (0.28, 0.64)**	**0.58 (0.36, 0.92)**	**0.43 (0.28, 0.67)**

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Risk factor	Control	Case	Univariate OR (95% CI)	Multivariable OR1 (95% CI)*	Adjusted OR2 (95% CI)^
Linear trend			**0.77 (0.69, 0.86)**	**0.85 (0.75, 0.95)**	**0.77 (0.69, 0.86)**

*All risk factors listed in the table except for total number of variants were included in the multivariable model (multivariable OR1); age-sex corrected log body nevus number and presence of atypical nevi were adjusted in OR1 for total number of variants;

^adjusted for age, sex, hair color, freckling as child, general skin type without other nevus phenotype or genotype.

Table 4

Association between nevus phenotype and genotype and melanoma risk by tumor site

Risk factor	Control	Trunk	OR (95% CI)	Limbs	OR (95% CI)	Head & neck	OR (95% CI)	Rare sites§	OR (95% CI)**	All sites	OR (95% CI)
Residual log body nevus number quartile *											
Q1	126 (25)	12 (4)	-	23 (5)	-	18 (15)	-	2 (4)	-	55 (6)	-
Q2	126 (25)	41 (13)	**3.47 (1.65, 7.32)**	51 (12)	**2.59 (1.43, 4.69)**	25 (21)	1.72 (0.82, 3.60)	10 (18)	-	127 (14)	**2.64 (1.71, 4.08)**
Q3	126 (25)	68 (21)	**5.48 (2.68, 11.18)**	92 (21)	**4.90 (2.78, 8.64)**	31 (26)	**2.14 (1.05, 4.38)**	14 (25)	**3.31 (1.31, 8.39)**	205 (22)	**4.32 (2.84, 6.59)**
Q4	126 (25)	202 (63)	**17.58 (8.86, 34.85)**	274 (62)	**13.32 (7.78, 22.80)**	45 (38)	**3.61 (1.81, 7.22)**	30 (54)	**6.42 (2.81, 14.66)**	551 (59)	**11.66 (7.78, 17.48)**
Presence of atypical nevi *											
0	462 (92)	198 (61)	-	316 (72)	-	100 (84)	-	43 (77)	-	657 (70)	-
1+	42 (8)	125 (39)	**2.27 (1.44, 3.59)**	124 (28)	**2.07 (1.31, 3.27)**	19 (16)	1.46 (0.73, 2.94)	13 (23)	1.82 (0.73, 4.55)	281 (30)	**2.08 (1.41, 3.06)**
rs12203592_chr6 ^											
CC	279 (57)	220 (70)	-	273 (63)	-	71 (60)	-	34 (63)	-	598 (65)	-
CT	185 (38)	78 (25)	**0.54 (0.39, 0.74)**	141 (31)	0.78 (0.59, 1.03)	41 (35)	0.87 (0.57, 1.34)	19 (35)	0.84 (0.47, 1.52)	279 (30)	**0.70 (0.56, 0.89)**
TT	22 (5)	15 (5)	0.87 (0.44, 1.71)	20 (5)	0.93 (0.50, 1.74)	6 (5)	1.07 (0.42, 2.74)	1 (2)	0.37 (0.05, 2.86)	42 (5%)	0.89 (0.52, 1.52)
Linear trend			**0.68 (0.53, 0.88)**		0.85 (0.68, 1.07)		0.94 (0.66, 1.33)		0.77 (0.46, 1.29)		**0.80 (0.66, 0.96)**
rs7023329_chr9 ^											
GG	94 (20)	90 (30)	-	115 (28)	-	29 (25)	-	16 (31)	-	250 (29)	-
GA	274 (58)	146 (49)	**0.56 (0.39, 0.79)**	209 (51)	**0.62 (0.45, 0.86)**	61 (54)	0.72 (0.44, 1.19)	25 (49)	0.54 (0.27, 1.05)	441 (50)	**0.61 (0.46, 0.80)**
AA	107 (23)	61 (21)	**0.60 (0.39, 0.91)**	89 (22)	0.68 (0.46, 1.01)	24 (21)	0.73 (0.40, 1.34)	10 (20)	0.55 (0.24, 1.27)	184 (21)	**0.65 (0.46, 0.90)**
Linear trend			**0.76 (0.61, 0.94)**		**0.82 (0.67, 1.00)**		0.85 (0.62, 1.16)		0.71 (0.46, 1.11)		**0.80 (0.68, 0.95)**
rs2284063_chr22 ^											
GG	193 (40)	148 (49)	-	194 (46)	-	56 (47)	-	23 (43)	-	421 (47)	-
GA	226 (47)	126 (41)	**0.73 (0.54, 0.99)**	187 (44)	0.82 (0.62, 1.09)	57 (48)	0.87 (0.57, 1.32)	26 (48)	0.97 (0.53, 1.75)	396 (44)	0.80 (0.63, 1.02)
AA	66 (14)	31 (10)	**0.61 (0.38, 0.99)**	45 (11)	0.68 (0.44, 1.04)	6 (5)	**0.31 (0.13, 0.76)**	5 (9)	0.64 (0.23, 1.74)	87 (10)	**0.60 (0.42, 0.87)**
Linear trend			**0.76 (0.62, 0.95)**		**0.82 (0.68, 1.00)**		**0.69 (0.50, 0.95)**		0.85 (0.56, 1.30)		**0.79 (0.67, 0.93)**

§ Rare sites include acral, nodal with no known primary, anal, buttock, foot, sub-ungual, vulval;

* OR adjusted for age, sex, hair color, freckling as child, general skin type, and (for atypical nevi only) residual log body nevus number;

^ crude ORs reported;

** quartile 1 and quartile 2 were grouped together in the comparison of residual log body nevus number for rare tumor sites.

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Table 5

Association between nevus phenotype and genotype and melanoma risk by age subgroup

Risk factor	Age < 50				Age ≥ 50			
	Control	Case	OR (95% CI)	P trend	Control	Case	OR (95% CI)	P trend
Residual log body nevus number quartile *								
Q1	45 (31%)	26 (7%)	-		81 (23%)	29 (5%)	-	
Q2	30 (21%)	55 (14%)	3.08 (1.51, 6.28)		96 (27%)	72 (13%)	2.59 (1.46, 4.59)	
Q3	34 (24%)	91 (23%)	4.48 (2.28, 8.79)		92 (26%)	114 (21%)	4.44 (2.54, 7.74)	
Q4	35 (24%)	224 (57%)	10.27 (5.38, 19.62)	<0.0001	91 (25%)	327 (60%)	13.34 (7.76, 22.94)	<0.0001
Presence of atypical nevi *								
No	125 (87%)	250 (63%)	-		337 (94%)	407 (75%)	-	
Yes	19 (13%)	146 (37%)	1.64 (0.90, 3.01)	0.11	23 (6%)	135 (25%)	2.33 (1.39, 3.91)	0.001
rs12203592_chr6 ^								
CC	76 (55%)	250 (65%)	-		203 (58%)	348 (65%)	-	
CT	55 (40%)	116 (30%)	0.64 (0.43, 0.97)		130 (37%)	163 (30%)	0.73 (0.55, 0.98)	
TT	6 (4%)	18 (5%)	0.91 (0.35, 2.38)	0.11	16 (5%)	24 (4%)	0.88 (0.45, 1.69)	0.08
rs7023329_chr9 ^								
GG	30 (22%)	105 (29%)	-		64 (19%)	145 (29%)	-	
GA	73 (54%)	193 (52%)	0.76 (0.46, 1.23)		201 (59%)	248 (49%)	0.55 (0.39, 0.77)	
AA	33 (24%)	70 (19%)	0.61 (0.34, 1.08)	0.09	74 (22%)	114 (22%)	0.68 (0.45, 1.03)	0.06
rs2284063_chr22 ^								
GG	50 (36%)	182 (48%)	-		143 (41%)	239 (46%)	-	
GA	64 (46%)	164 (43%)	0.70 (0.46, 1.08)		162 (47%)	232 (44%)	0.86 (0.64, 1.14)	
AA	24 (17%)	35 (9%)	0.40 (0.22, 0.74)	0.003	42 (12%)	52 (10%)	0.74 (0.47, 1.17)	0.15

*OR adjusted for age, sex, hair color, freckling as child, general skin type, and (for atypical nevi only) residual log body nevus number;

^crude ORs reported. There was no statistically significant difference in melanoma risk between age groups for the nevus genotypes (p=0.76, 0.75 and 0.12 for SNPs on chromosomes 6, 9 and 22, respectively).